

## ***Tubifex tubifex* culturing Standard Operating Procedure**

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Culture conditions: 23±2°C, photoperiod 18:6 h (light:dark)

### **Culture substrate/water/container**

1.625 L of sieved (< 250 µm) quartz sand (Repti-sand, Zoo Med Labs, Inc., www.zoomed.com).

3.375 L of dechlorinated water (pH 7-8.5, continuous gentle aeration DO 80-100%).

4.5 L (10 X 25 X 25 cm) plastic container (Rubbermaid) (0.063 m<sup>2</sup> sand surface area)

### **Food**

Combination of iceberg lettuce and flake fish food

Recipe (makes ~800-1000 ml):

Ingredients: head of iceberg lettuce, 10 g (dw) dry flake fish food (Tetra-min or equivalent)

1. Take apart lettuce head and rinse leaves in distilled water.
2. Add ~100 ml (1/2 cup) of distilled water and fish food to standard kitchen blender.
3. While blending (low speed), slowly add lettuce leaves until all lettuce blended.
4. Blend on high for 5 minutes.
5. Transfer aliquots of the mixture to 50-ml plastic vials or centrifuge tubes, freeze and store at < 0°C.

### **Culture types**

**Mass culture:** Worms from all life stages, mainly adults for sustaining culture populations and production of cocoons for beginning age-specific cultures.

**Age specific culture:** Started with 150 cocoons\*, produces ~100 8-week old adults for toxicity testing.

Mass culture maintenance schedule:

<u>Time (week)</u>	<u>Activity</u>
0	Start culture with 200-400 adults and a few cocoons, 50 ml food mixed into sand before addition of water
2	Add 50 ml food
4	Change overlying water, add 50 ml food
6	Add 50 ml food
8	Sieve sand (500 µm), save adults and/or cocoons for other cultures, wash sand with detergent and save for reuse

Age-specific culture maintenance schedule:

<u>Time (week)</u>	<u>Activity</u>
0	Start culture with 150 cocoons**, 25 ml food mixed into sand before addition of water
2	Add 25 ml food
4	Change overlying water, add 50 ml food
6	Add 50 ml food
8	Sieve sand (500 µm), save 8-week old adults for use in toxicity test, wash sand with detergent and save for reuse

- \* Each cocoon added will produce approximately 0.6 8-week old adults (all of the cocoons do not hatch, there is some mortality, etc.). Can start cultures with up to 400 cocoons (possibly more) if necessary, without affecting the size or apparent health of the resultant 8-week old adults.
  
- \*\* "Planting" cocoons: Setup culture (with sand, food, water) and place in culturing chamber. Add cocoons to culture and lightly cover most of the cocoons with sand (it's okay for some to be on the surface). Do not bury the cocoons deeper than ~0.5-1 cm (?) or they will not hatch.